ORIGINAL ARTICLE

Antimicrobial effect of 2% chlorhexidine as a chemical adjuvant in different endodontic protocols: an in vitro study

ABSTRACT

Aim: *To analyze the antimicrobial effect of different protocols using 2% chlorhexidine as an irrigating substance, and 2.5% sodium hypochlorite to decontaminate lower molars infected with Enterococcus faecalis.*
Methodology*: 72 mesial roots were sectioned and contaminated with E. faecalis. The samples were randomly distributed into 4 groups (n=14) according to the protocols: 2 ml of 2% chlorhexidine gel and 10 ml of 9% saline solution (CHX G+SS); 2 ml of 2% chlorhexidine gel and 10 ml of 2% liquid chlorhexidine (CHX G+CHX L); 12 ml of 2% liquid chlorhexidine (CHX L); 12 ml of 2.5% liquid sodium hypochlorite (HIP L) (positive control). Bacteriological samples were collected before preparation and irrigation (S1), and after instrumentation and irrigation with different protocols (S2), for the ultimate purpose of quantifying the reduction in planktonic bacteria and intracanal biofilm. The samples were evaluated by using scanning electronic microscopy (SEM) to confirm the presence of biofilm. Bacterial quantification was performed using qPCR and the colony forming unit (CFU)/mL count. Statistical analysis was performed using the Kruskal-Wallis and Wilcoxon tests to compare the protocols of use for chlorhexidine as a according to the time points tested. The Student-Newman-Keuls test was used for multiple comparisons, with a significance level of 5%.*
Results: *The SEM analysis allowed visualizing the biofilm structure. At S1, there was a significant difference among the teeth that made up each group (p<0.001) regarding the CFU count. At S2, there was no difference among the HIP L, CHX G+CHX L and only CHX L groups, but the CFU count was significantly higher in the CHX G+SS group (p<0.001). Significantly lower CFU counts were found after S2, for all the groups (p=0.010).*
Conclusion: *The application of different 2% chlorhexidine protocols was effective in reducing bacterial contamination by E. faecalis. The 2% chlorhexidine application protocols proved to be good alternatives to 2.5% sodium hypochlorite, given the excellent antimicrobial efficacy.*

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Introduction

Endodontic treatment has a high success rate (1), but can fail when confronted with anatomical difficulties (Ahmed et al. 2017) and microbial contamination (Siqueira et al, 2018). Chemical-mechanical preparation (CMP) is performed to decontaminate the root canal system and promote the healing and repair of periapical tissues (Bergenholtz et al. 2016). Endodontic failure is associated with the persistence of viable microorganisms even after endodontic treatment (Siqueira et al. 2008, Nardello et al. 2022). Among the microorganisms commonly found in cases of endodontic failure, *E. faecalis* has been isolated frequently and presents important factors of virulence and microbial resistance (Sundqvist et al 1998, Williams et al 2006, Saatchi et al. 2014).

The endodontic instruments used in preparing root canals are often incapable of completely disorganizing the biofilm on the walls of the root canal (Martinho et al. 2014, Soares et al. 2018), especially in anatomical regions of difficult access, such as isthmuses, lateral canals, deltas, and apical and dentinal tubules (Marinho et al 2015, Siqueira et al. 2008). In this case, irrigating solutions are recommended to remove the debris, thereby dissolving organic matter, disinfecting the root canal system, and lubricating the instruments during endodontic preparation (Haapasalo et al. 2014).

The most widely indicated and used irrigating substance in an endodontic clinic is sodium hypochlorite. This substance has excellent antimicrobial properties, is capable of disorganizing the biofilm, and has tissue dissolution ability (Naenn et al 2004, Mohammadi et al. 2013, Solana et al. 2017). However, its efficacy is dependent on volume, concentration and duration of contact with the organic matter (Macedo et al. 2010, Fedorowicz et al., 2012, Boutsioukis et al. 2022); moreover, it displays cytotoxicity when it comes into contact with periapical tissues, mainly at higher concentrations (Holland et al 1992, Tanomaru et al 2002, Gernhardt et al. 2004).

Chlorhexidine digluconate has been proposed as an alternative to Sodium hypochlorite, because it also has favorable antimicrobial properties and substantivity (Gomes et al.2013).

Chlorhexidine is biocompatible and effective against gram positive and gram negative microorganisms (Gomes et al. 2013, Roças et al 2016, Vianna et al. 2004); however, it also has drawbacks, such as not dissolving organic matter, and not neutralizing bacterial liposaccharides (LPS) (Gomes et al. 2013).

Both sodium hypochlorite and chlorhexidine have been reported in several studies as efficient against microorganisms, including *E. faecalis* (Ruksakiet et al. 2020, Estrela et al. 2008). However, several clinical studies have demonstrated the efficacy of chlorhexidine (Gomes et al. 2013) but point out that gaps still exist regarding an efficient protocol of use. Authors have reported its use as an antimicrobial at a 2% concentration in liquid form (Fedorowicz et al. 2012, Zandhi et al. 2009); however, there are studies proposing its use at 0.2% (Ringel et al. 1982), 0.12% (Siqueira & Roças 2011), and 2% concentration in gel form (Gomes et al, 2009). The gel form can be used together with saline solution for irrigation, thus taking advantage to lubricate endodontic instruments and benefit from the gel's rheological action. Some authors have used the gel form in a clinical study protocol (Vianna et al. 2006, Gomes et al. 2009, Marinho et al. al, 2015).

Considering the possibilities of different irrigating solutions, and different protocols found in the literature, this study aimed to evaluate the antimicrobial effect of different protocols using 2% chlorhexidine as a irrigating substance to decontaminate lower molars infected with *E. faecalis*. The null hypothesis was that the application of different protocols for using 2% chlorhexidine as an irrigating substance solution would not alter the bacterial contamination in the root canal.

Material and Methods

Tooth Selection

This study was submitted to approval by the institutional ethics committee (CAAE4.

539.304). A total of 72 mandibular and maxillary molars that met the inclusion criteria were selected from 150 mandibular teeth donated to the study. The sample calculation was conducted using the G Power 3.1.9.4 program, adopting the analysis of variance model. An effect size of 0.459 was obtained from the results presented by Dametto et al. (2005), using a significance level of 5% and power of 80%. The sample calculation indicated that 14 mesial roots of human lower molars were needed in each of the groups, for a total of 72 mesial roots. A total of 56 samples were distributed into 4 groups (n=14). 8 samples were also selected for SEM evaluation, 4 samples for positive control and 4 samples for negative control.

The teeth were radiographed mesiodistally and buccolingually, and observed under the optical lens of a clinical microscope (ALL 001, Alliance Microscopy, São Carlos, SP, Brazil), at 20x magnification to determine whether they qualified for the inclusion criteria, and which mesial roots of mandibular first and second molars had separate mesiobuccal (MV) and mesiolingual (ML) canals with independent foramina, fully formed apices, and roots with an anatomical foramen diameter compatible with a #15 K hand file (C-Pilot, VDW, Munich, Germany). Only teeth with a maximum degree of curvature of 10° to 20° were selected, according to Schineider's classification (1971), and teeth with similar canal volume, dentin surface area and foraminal diameter. Presence of internal and external resorptions, calcifications, root cracks, fractures or previous endodontic treatment were considered exclusion criteria.

Tomographic analysis

The anatomical characteristics of the researched teeth were analyzed by cone beam computed tomography (CBCT). CBCT images were acquired using a Carestream 9600 unit operating at 85 kVp and 6 mA, with an exposure time of 14 s (Carestream Dental, Atlanta, GA, USA) of these seconds with an 8x8 cm FOV and 0.1 mm voxel size. The analysis was performed using the GALAXIS 3D software (Sirona Galileos, Bensheim, Germany).

Sample Preparation

The teeth were disinfected with 5% sodium hypochlorite in immersion for 1h, and stored in thymol at room temperature (Berutti, 2013). Root surfaces were cleaned with periodontal curettes (Golgran Instrumental Odontológicos, São Caetano do Sul, Brazil). Coronary access was performed with a high-speed drill under refrigeration, and the roots were separated with a diamond disc (KG Sorensen, São Paulo, Brazil). The root length was standardized at 17 mm (Stringheta et al., 2019; Zuolo et al., 2017). The working length was standardized exactly to the measure of 0.0, tangent to the apical foramen (real working length=root canal real length - RL).

The root canals were irrigated with 5 ml of 2.5% sodium hypochlorite (Formula e Ação, São Paulo, SP, Brazil) followed by preparation of the canal with a Prodesign Logic file 03/15 (Easy, Belo Horizonte, Minas Gerais) up to the RL. Subsequently, the root canals were filled with 17% ethylenediaminetetraacetic solution (EDTA, Biodinamica São Paulo, Brazil). The solution was stirred with an ultrasound device (Satelec Booster, Brazil), and inserted (PN43807; Satelec Booster; Acteon, Indaiatuba, SP, Brazil) for 1 minute inside the root. Final irrigation was performed with 5 ml of sterile distilled water. Afterwards, the root canals were dried with #15 paper cones (Dentsply Maillefer, Ballaigues, Switzerland). The apical foramen of the roots was sealed with Z 100 composite resin (3M, St. Paul, MN), according to the manufacturer's protocol, and the external surface was waterproofed with nail polish (Colorama, São Paulo, Brazil). The samples were sterilized in an autoclave at 121 °C for 30 minutes (Cristófoli, Campo Mourão, Brazil).

Contamination of samples with *E. faecalis*

E. faecalis (ATCC-29212) was cultured and stored in brain heart infusion (BHI) broth media with 20% glycerol. The inoculum was prepared by transferring 100 µL of the *E. faecalis* stock to 2 mL of BHI broth, and storing it in a lab incubator at 37 °C. The sterilized roots were contaminated with a micropipette (Kasvi, Curitiba, Paraná, Brazil). Twenty µL of the final concentration of the *E. faecalis* suspension was placed inside

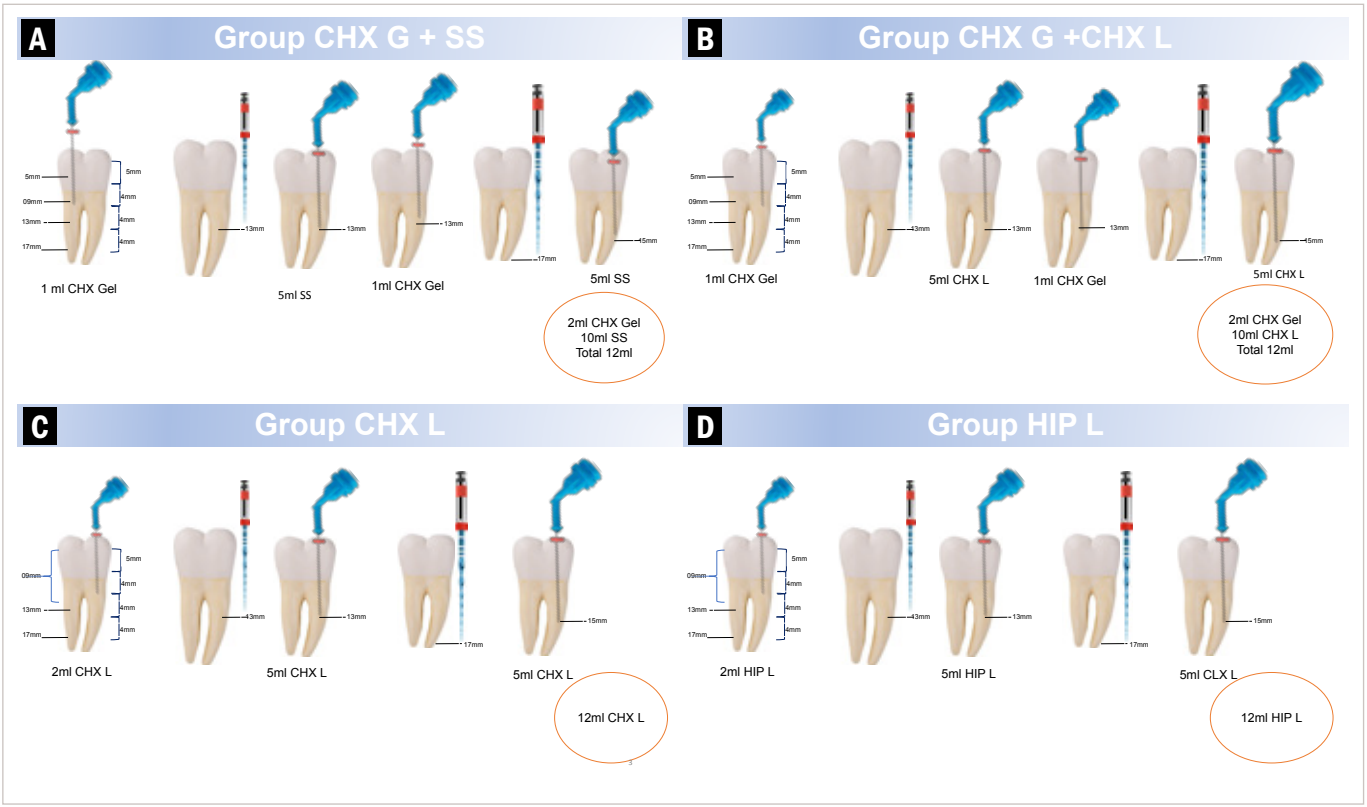

Figure 1
Irrigation protocols: **(A)** 2% chlorhexidine gel and 9% saline solution (CHX G+SS), **(B)** 2% chlorhexidine gel and 2% liquid chlorhexidine (CHX G+CHX L), **(C)** 2% liquid chlorhexidine (CHX L) and **(D)** 2.5% liquid sodium hypochlorite (HIP L).

the root canals. Next, the samples were stored in a lab incubator (Tecnal-Equipamentos para Laboratórios, Piracicaba, SP, Brazil) at 37 °C with 5% CO_2 for four weeks. Confirmation of the viability and purity of the microorganisms inside the canals was carried out weekly by randomly collecting two teeth, seeding them in BHI broth, incubating them in a lab incubator at 37 °C with 5% CO2 for 24 hours, and applying the Gram stain.

Root Canal Prepare and Irrigation
Instrumentation in the cervical, middle and apical thirds was performed with a Reciproc Blue 25/08 file (VDW, Munich, Germany) and a Gold Reciproc motor (VDW, Munich, Germany) with penetration and traction movements, following the manufacturer's recommendations.
The samples were divided into four experimental groups (n=14), plus a positive control and a negative control. Eight samples were evaluated by scanning electronic microscopy (SEM) to confirm the presence of biofilm. The teeth were divided into groups, according to the irrigation protocol represented in Figure 1:

• Group CHX G+SS: irrigation with 2 ml of 2% chlorhexidine gel and 10 ml of saline solution;
• Group CHX G+CHX L: irrigation with 2 ml of 2% chlorhexidine gel and 10 ml of 2% liquid chlorhexidine;
• Group CHX L: irrigation with 12 ml of 2% liquid chlorhexidine;
• Group HIP L (positive control): irrigation with 12 ml of 2.5% sodium hypochlorite;
• Group CN (negative control): contaminated and non-irrigated samples (no treatment);
• Group CE (sterilization control): samples sterilized and not contaminated.
Single-use Reciproc files were used according to the manufacturer's protocol. Mechanical preparation of root canals was performed by a single operator under sterile conditions in a laminar flow cabinet.

Quantification of E. faecalis contamination
Bacterial collection was performed after four weeks of initial contamination of the samples (S1). Aliquots of 0.1 ml of the suspension, together with each dilution (10^{-2}, 10^{-4}, 10^{-5} and 10^{-6}), were seeded in Petri dishes (CRAL Artigos para Laboratório, Cotia, SP, Brazil) contain-

ing BHI agar (KASVI, Curitiba, PR, Brazil), and incubated in a lab incubator with 5% CO2 at 37 °C for 24 hours. Subsequently, the number of colony forming units (CFUs) per plate was counted, the number of CFU/mL was calculated, and quantitative analysis was performed by real time quantitative polymerase chain reaction (qPCR). Immediately after concluding the instrumentation, the final collection (S2) was made with a sterilized #15 Hedstrom file, introduced inside the root canal in the RL. Serial dilutions (10^{-2}, 10^{-4}, 10^{-5} and 10^{-6}) were prepared from the suspension. Aliquots of 0.1 ml of the suspension together with each dilution were plated in Petri dishes (CRAL Artigos para Laboratório, Cotia, SP, Brazil) containing BHI agar (KASVI, Curitiba, PR, Brazil). The seeded plates were incubated in a lab incubator with 5% CO2 at 37 °C for 24 hours. Subsequently, the number of CFUs per plate was counted, and the number of CFU/ml was calculated.

Quantitative analysis by qPCR
DNA was extracted from half of the sample volume using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA extracts were frozen at 20 °C until qPCR analysis. *E. faecalis* cells in root canal samples were quantified using the qPCR method targeting the 16S rRNA gene, with the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on an ABI 7500 real-time PCR instrument (Applied Biosystems) with a total reaction volume of 20 µL. Specific primers for *E. faecalis* species were used according to a previous study (Siqueira & Roças 2004). An accumulation of the PCR product was detected at each cycle by monitoring the increased fluorescence of the dye (dsDNA-binding SYBR Green). All measurements were performed in duplicate for samples, and in triplicate for standardization. Data acquisition and analysis were performed using the ABI 7500 v2.0.4 software (Applied Biosystems).
E. faecalis ATCC 29212 was used to create a 10 log standard curve for direct bacterial quantification. DNA was isolated from a pure fresh culture of this strain using the QIAamp DNA Mini Kit (Qiagen), and quantified using a spectrophotometer (BioPhotometer, Eppendorf, Hamburg, Germany). The DNA value measured was converted into target genomic copy levels per microliter, by using the formula

$$m=n\ [1\ mole\ /6 \cdot 1023\ (bp)]\ [660\ (g)\ /\ mole]$$
$$=n\ [1.096\ x\ 10\text{-}21\ (g)\ /bp)],$$

where m is the genomic mass of a single cell, and n is the genome size. Genome copying levels were considered numerically equivalent to bacterial cell levels. The standards were then diluted 10 times from 107 to 102 cells in TE buffer, and used to construct the standard curve.

Scanning Electron Microscopy (SEM)
Two roots from each group were randomly chosen and fixed in 10% buffered formalin for one week. Next, the prepared root canal walls were analyzed and topographically evaluated by coronal, medium and apical thirds with SEM (JSM 5600 LV; JEOL, Tokyo, Japan), at a voltage acceleration of 15 kV, with magnifications of 5,000x and 10,000x to confirm bacterial colonization and permanence of the biofilm. The roots were divided longitudinally, mounted on an acrylic stub, and covered by deposition of gold metal ions (sputtering), in a metalizing machine, prior to SEM analysis.

Statistical analysis
Comparisons between the protocols of chlorhexidine used as an irrigating substance-and the evaluation time points were made using Kruskal-Wallis and Wilcoxon tests, following non-normality of the CFU count data to normal distribution and homogeneity of variance. The Student-Newman-Keuls test was used for multiple comparisons. Statistical calculations were performed using the SPSS 23 (SPSS, Chicago, IL, USA), and BioEstat 5.0 (Mamirauá Foundation, Belém, PA, Brazil) programs, at a 5% significance level.

Results

The SEM analysis allowed viewing the morphology of the root canals and the bio-

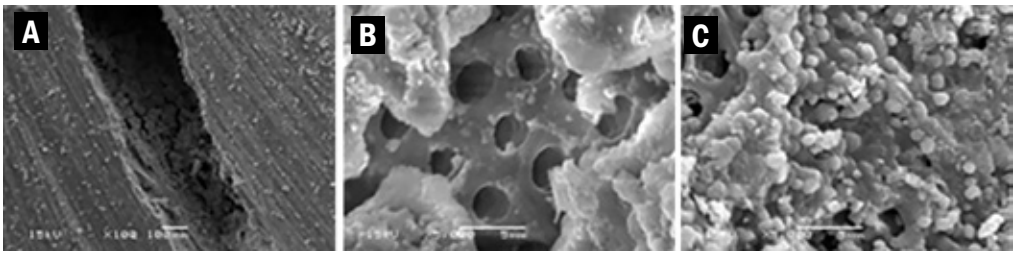

Figure 2
(A) Cutting the root – root canal, distant view (SEM at 100x). **(B)** Root of the control group (SEM at 5000x). **(C)** Bacterial contamination in the root canal (SEM at 5000x).

film through the section of the roots (Fig. 2A). Fig. 2B was taken from the root canal of the negative control group. It demonstrates the absence of bacteria-like structures, and only the dentinal tubule entrance and amorphous structures that can be identified as debris remains. Fig. 2C also shows the presence of a circular structure similar to *E. faecalis*. Since this genus of bacteria commonly presents ovoid or circular morphology (García-Solache et al., 2019), this may represent the presence of bacterial contamination in root canals, and hence biofilm formation.

At S1, there was a statistically significant difference in the CFU count among the teeth that made up each group ($p<0.001$). The group that was to be submitted to the irrigation protocol with 2.5% liquid sodium hypochlorite had a significantly higher number of CFUs than the other irrigation groups. In the group whose irrigation was to use 2% liquid chlorhexidine (CHX L), the initial microbiological count did not differ significantly from that found in any of the other three groups of teeth (Table 1).

After instrumentation and irrigation (S2), the protocols showed a statistically significant effect on the microbiological count ($p<0.001$). There was no difference between the groups treated with the irrigation protocols performed with 2.5% liquid sodium hypochlorite, 2% chlorhexidine gel and liquid, and only 2% liquid chlorhexidine, but the CFU count in the group that received 2% chlorhexidine gel and 9% saline solution was significantly higher (Table 1). Significantly lower CFU counts were found in all the groups after instrumentation and irrigation ($p=0.010$), and even in the groups that had no CFUs, but that received 2.5% liquid sodium hypochlorite, 2% chlorhexidine gel and liquid, and only 2% liquid chlorhexidine (Table 1). A significant difference was also found between the irrigation protocols in regard to the absolute reduction of the CFUs ($p<0.001$). The number of CFUs for the 2.5% liquid sodium hypochlorite group and the 2% liquid chlorhexidine group was significantly lower compared to the group that received 2% chlorhexidine gel and liquid. As for the number of CFUs for 2% chlorhexidine gel and 9% saline solution, there was no significant difference between the group irrigated with 2% liquid chlorhexidine and that receiving 2% chlorhexidine gel and liquid irrigation. However, the protocol using 2% chlorhexidine gel and 9% saline solution promoted a significantly higher number of CFUs, compared with the protocol for 2.5% liquid sodium hypochlorite (Table 1).

Discussion

Endodontic failure is directly related to the perseverance of viable microorganisms after endodontic intervention (Siqueira et al.2018). *E. faecalis* is significantly associated with persisting endodontic infections, and is found in 24% to 77% of teeth with endodontic treatment failures (Vidana et al., 2011). Therefore, this study adopted contamination with *E. faecalis* to simulate a clinical situation that could be used to evaluate the potential of irrigation protocols to resolve these infections. The evaluation of SEM images ensured the methodology chosen for contaminating the samples and revealed the biofilm formation (Fig. 2).

Several irrigation protocols with endodontic solutions have been proposed in the literature to enhance the mechanical cleaning of endodontic instruments (Haapasalo et al. 2014). Among these solutions, sodium hypochlorite (NaOCl) has been advocated for its high antimicrobial activity, especial-

Table 1

Means, standard deviations, medians and mean order of number and reduction of colony forming units (CFU/mL), before and after instrumentation and irrigation with different chlorhexidine use protocols.

Group	Collection time		Absolute reduction	Percent reduction
	S1	S2		
CHX G+SS	554,790 (686,243) Med: 306,000^{Aa}	8,874 (14,465) Med: 2,887^{Bb}	545,916 (685,851) Med: 277,933 Mean ord: 31.6^{BC}	95.9% (4.7%) Med: 98.3%
CHX G+CHX L	165,810 (141,171) Med: 111,333^{Aa}	0 (0) Med: 0^{Ab}	165,810 (141,171) Med: 111,333 Mean ord: 42.4^{C}	100.0% (0.0%) Med: 100.0%
CHX L	584,286 (322.162) Med: 573.333^{Aba}	0 (0) Med: 0^{Ab}	584,286 (322,162) Med: 573,333 Méd. ord: 21,9^{AB}	100.0% (0.0%) Med: 100.0%
HIP L	905,143 (757,758) Med: 606.667^{Ba}	0 (0) Med: 0^{Ab}	905,143 (757,758) Med: 606,667 Méd. ord: 18,2^{A}	100.0% (0.0%) Med: 100.0%

Standard deviation in parentheses. Med=median. Average order=average of the orders. Medians or mean orders followed by distinct capital letters indicate a significant difference between the groups (comparisons within each column). Medians followed by equal lowercase letters indicate no significant difference between the counts before or after instrumentation and irrigation. Chlorhexidine gel 2% and saline 9% (CHX G+SS), chlorhexidine gel 2% and chlorhexidine liquid 2% (CHX G+CHX L), liquid chlorhexidine 2% (CHX L) and sodium hypochlorite 2.5% (HIP L).

ly against *E. faecalis* (Dáviz et al. 2020). Nevertheless, it has limited action, and its efficacy is dependent on volume and concentration (Macedo et al. 2010). Although high concentrations are efficient, they pose the risk of tissue toxicity (Gernhardt et al 2004). For this reason, 2% chlorhexidine has been proposed in the literature by several authors, with the aim of exploring its safe biological properties, and antimicrobial ability (Gomes et al. 2009). Therefore, this study sought to evaluate *E. faecalis* decontamination with different chlorhexidine formulations. Furthermore, this study uses irrigating substances at each change in the use of endodontic files, in different groups, simulating clinical use, as well as other studies (Siqueira et al, 2018).

Significantly lower results were obtained for reduced CFUs after applying the 2% chlorhexidine protocols (p=0.010), absolute reduction was observed in groups 1, 2 and 3. Therefore, the null hypothesis was rejected. All the groups showed a significant reduction in CFUs, with the CHX L and HIP L groups showing the lowest decrease in CFUs (p<0.001), but not differing from each other. This corroborates the result of studies that have demonstrated the efficacy of chlorhexidine (CHX) and NaOCl on *E. faecalis* (Estrela et al. 2008). Zand et al. compared the efficacy of CHX with that of NaOCl in reducing endodontic infection and obtained similar results for both solutions (Zand et al., 2010). In 2020, a systematic review and Meta-analysis by Ruksaki-

et et al. also compared the effectiveness of CHX and NaOCl as antimicrobials and found no statistical difference between the irrigants. These articles show the good antimicrobial potential of both CHX and NaOCl. As for the difference between the formulations and the protocols, CHX gel (CHX G+SS) and CHX L showed no statistical difference in the absolute reduction of CFUs ($p<0.001$), even though the group with saline solution showed a higher number of remaining CFUs than the other groups ($p<0.0001$). The advantage of using the gel form is that there is less debris extrusion and smear layer reduction, compared with CHX L solutions, because of the properties of viscosity and rheological action (Arruda-Vasconcelos et al., Ferraz et al.2001). These properties can be favorable, despite the non-dissolution of organic tissues, and have substantivity, which causes CHX to have residual effects (Zand et al. 2010, Ferraz et al. 2001, Gomes at al. 2013).

The combination of CHX gel and saline solution (CHX G+SS) is proposed as a clinical protocol (Ferraz et al. 2007), for the purpose of improving the fluidity of the material, and was adopted as a protocol for this study. The irrigation dynamics vary according to physical parameters, such as flow velocity, wall shear stress, turbulence and apical pressure, and cause the biofilm to adhere to the root canal, and the debris and smear layer to be detached (Sujith et al 2021). The irrigant must be fluid when pressed out of the syringe, so that it offers less resistance to the flow, and thus comes into contact with the dentinal tubules in the root canal system to decontaminate them (Basrani et al. 2004). When the irrigant is used as a gel, it cannot penetrate deep enough inside the tubules to promote an antimicrobial effect (Zand et al. 2016). Therefore it causes a slight lower microbial effect. This suboptimal effect could account for the CHX G+SS having removed less bacteria than the groups in which CXH L had bacterial action (CFU count was reduced by 98.3%, versus 100% for the other groups.

Evaluations of CHX are found in the literature as well as NaOCl gel, Zand et al. (2016) found significantly higher antimicrobial activity against *E. faecalis* for 2.5% hypochlorite in liquid form than gel form (Zand et al., 2016). The authors mention that the lower antimicrobial ability of NaOCl may be related to the viscosity of the gel, which impairs its penetration into the dentinal tubules. The same may occur with CHX gel. When associated only with saline solution, it showed less CFU removal, but this did not occur when it was combined with liquid CHX irrigation, in which case the liquid CHX penetrated the dentinal tubules and imparted antimicrobial action to the gel form.

A limitation in the methodology of this study was the inability to supply all the groups with the same number of bacteria to compare the difference between contamination prior to instrumentation and to irrigation. A statistical difference in the CFU count was found among the teeth that made up each group ($p<0.001$). However, the difference in the initial contamination among the groups does not interfere with the objective of the study, which was to analyze the antimicrobial effect after different protocols of use of 2% CHX as an irrigating substance. Thus, the decrease in contamination within each group was taken into account ($p<0.001$).

It is noteworthy that this investigation was an ex vivo study, and differs from a clinical situation. The literature has reported on infections consisting of predominantly facultative anaerobes and gram-positive species that resist endodontic treatment; however, the most commonly found microorganism in cases of retreatment is *E. faecalis* (Barbosa-Ribeiro et al., 2021). Thus, further in vivo studies should evaluate the antimicrobial effect of 2% CHX as an irrigating substance, as well as long-term clinical follow-ups.

Conclusion

Different protocols of 2% chlorhexidine both in gel and liquid formulations, and of sodium hypochlorite, are effective in decontaminating root canals infected with *Enterococcus faecalis*, demonstrating that the reduction of biofilm is significant for all groups tested.

Clinical Relevance

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Conflict of Interest

The authors deny any conflicts of interest related to this study.

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The authors affirm that we have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultant ships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript.

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